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Diminished degradation of yeast cytochrome *c* by interactions with its physiological partners

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ABSTRACT **The level and structure of yeast iso-1-cytochrome *c* and iso-2-cytochrome *c*, encoded by the nuclear genes *CYC1* and *CYC7*, respectively, are normally not altered in ρ^- mutants, which completely lack the cytochromes $a{\cdot}a_3$ subunits and cytochrome *b* that are encoded by mitochondrial DNA. In contrast, iso-cytochromes *c* containing the amino acid change Thr-78 → Ile (T78I) were observed at the normal or near-normal wild-type level in ρ^+ strains but were completely absent in ρ^- mutants. We have demonstrated with the "global" suppressor mutation Asn-52 → Ile and by pulse–chase labeling that the T78I iso-1-cytochrome *c* undergoes rapid cellular degradation in ρ^- mutants. Furthermore, specific mutations revealed that the deficiency of T78I iso-1 cytochrome *c* can be caused by the lack of cytochrome $a{\cdot}a_3$ or cytochrome c_1, but not by the lack of cytochrome *b*. Thus, this and certain other, but not all, labile forms of cytochrome *c* are protected from degradation by the interaction with its physiological partners.**

Iso-1-cytochrome *c* (iso-1) and iso-2-cytochrome *c* (iso-2) are encoded by the *CYC1* and *CYC7* nuclear genes, respectively (1, 2) and these isocytochromes *c* are found at 95% and 5%, respectively, of the total cytochrome *c* complement in derepressed cells of the yeast *Saccharomyces cerevisiae* (3). Early studies revealed that the levels and structures of both isocytochromes *c* generally are equivalent in ρ^+ and related ρ^- strains—i.e., mutants that completely or partially lack mitochondrial DNA and do not carry out mitochondrial protein synthesis. This finding is consistent with *CYC1* and *CYC7* being nuclear genes and suggested that the synthesis of the isocytochromes *c* is not significantly influenced by other mitochondrial deficiencies. In contrast, ρ^- strains are completely deficient in cytochromes *b* and $a{\cdot}a_3$, a result explained by the fact that mitochondrial DNA encodes cytochrome *b* and three of the subunits of cytochrome $a{\cdot}a_3$ (4).

The identification and characterization of the iso-2 structural gene, *CYC7*, in the yeast *S. cerevisiae* were initially carried out with the *CYC7-H1* and *CYC7-H3* mutations that caused overproduction of iso-2 because of abnormal promoters (2, 5, 6). The initial screens carried out in these studies relied on complete or partial loss of iso-2 in *CYC7-H1* ρ^- and *CYC7-H3* ρ^- strains, presumably due to alterations in the translated *CYC7* region. Although equivalent amounts of iso-2 were observed in ρ^+ and ρ^- strains having the normal iso-2 sequence, surprisingly about one-third of the *cyc7-H1* mutants had a greater deficiency in ρ^- compared with ρ^+ strains (7). In fact, iso-2 was completely deficient in some *cyc7-H1* ρ^- mutants, while iso-2 remained at the normal level in the corresponding *cyc7-H1* ρ^+ strain.

In this study, we have demonstrated that certain amino acid replacements caused iso-1 and iso-2 to become susceptible to degradation preferentially in ρ^- mutants. Furthermore, this increased sensitivity to degradation can arise by the absence of either cytochrome $a{\cdot}a_3$ or cytochrome c_1, the physiological partners of cytochrome *c*.

METHODS

Genetic Nomenclature. The symbols *CYC1*$^+$ and *CYC7*$^+$ denote, respectively, the wild-type alleles encoding iso-1 and iso-2 in the yeast *S. cerevisiae*, whereas *CYC1* and *CYC7* denote the corresponding loci and are generic symbols. Mutant alleles that produce either functional or nonfunctional forms of iso-1 are designated *cyc1* followed by the allele number (e.g., *cyc1-1195, cyc1-1196*, etc.). The symbols *CYC7-H1* and *CYC7-H3* denote mutations that caused overproduction of iso-2 because of abnormal promoters (2, 5, 6). The *cyc7-67* allele corresponds to a partial deletion that results in the loss of iso-2. The symbols *cyc7-H1-54* and *cyc7-H3-26*, for example, denote mutants of, respectively, *CYC7-H1* and *CYC7-H3*, containing either functional or nonfunctional forms of iso-2.

Oligonucleotides. Oligonucleotides used for transforming yeast were synthesized on an Applied Biosystems 380A DNA synthesizer.

Yeast Strains. The *cyc7-H1-54, cyc7-H1-56*, etc., mutants have been described by Downie *et al.* (7). A congenic series of strains was constructed from strain B-7528 (*cyc1-31 cyc7-67 ura3-52 lys5-10*) (8) or its derivatives (9). The following *cyc1* alleles encoding iso-1 with various amino acid replacements were constructed by transforming strains directly with synthetic oligonucleotides as described by Yamamoto *et al.* (9): *cyc1-1195* encoding iso-1 with Thr-78 → Ile (T78I) replacement, by transforming *cyc1-1011* with synthetic oligonucleotide OL92-80 (GAAATATATTCCTGGTATCAAGATGGCCTTTGGTGGGTTG); *cyc1-1197* encoding iso-1 with both T78I and Asn-52 → Ile (N52I) replacements, by transforming *cyc1-1051* with synthetic oligonucleotide OL92-80; *cyc1-1196* encoding iso-1 with the N52I replacement, by transforming *cyc1-1051* with synthetic oligonucleotide OL92-132 (GAAATATATTCCTGGTACCAAGATGGCCTTTGGTGGGTTG); and *cyc1-1233* encoding iso-1 with the Gly-41 → Arg (G41R) replacement, by transforming *cyc1-1010* with synthetic oligonucleotide OL92-149 (GTATCTTTGGCAGACACTCTAGACAAGCTGAAGGGTATTCGTA).

The ρ^+ were converted to ρ^- by growing the strains on a medium containing ethidium bromide (4).

A specific deficiency of cytochrome c_1 was produced by disrupting the structural gene *CYT1* (10, 11), whereas specific deficiencies of cytochrome $a{\cdot}a_3$ were produced by disrupting either *COX7*, which encodes subunit VII of cytochrome $a{\cdot}a_3$ (12), or *PET122*, which controls translation of the mitochondrial DNA-encoded subunit III (13). The disruptions were carried out by the conventional procedure of transforming cells with linear fragments having the coding regions of these genes replaced by the *URA3* marker. The linear fragments were prepared from the following plasmids: pKC206, which contained a 1.1-kb *Hin*dIII *URA3* fragment replacing a 225-bp

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Abbreviations: iso-1, iso-1-cytochrome *c*; iso-2, iso-2-cytochrome *c*.

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Acc I–*Sty* I fragment of *COX7*; pJD012, which contained a 1.1-kb *Hin*dIII *URA3* fragment replacing a 300-bp *Nru* I fragment of *PET122*; and pAB980, which contained a 1.1-kb *Hin*dIII *URA3* fragment inserted in the *Hin*dIII site of *CYT1*.

The congenic strains specifically lacking cytochrome *b* were isolated after crossing a [*cob*$^-$] *kar1* strain (JC-17-162) to each of the [ρ^-] strains (brackets refer to mitochondrial genotypes).

The *PIM1* (ref. 14), *YTA10* [ref. 15; also denoted *AFG3* (ref. 16)], *YME1* [ref. 17; also denoted *YTA11* (ref. 18)], and *RCA1* [ref. 19; also denoted *YTA12* (ref. 18)] genes were similarly disrupted with linear fragments prepared from the following plasmids: pAB984, which contained a 1.1-kb *Hin*dIII *URA3* fragment inserted in the *Hin*dIII site of *PIM1* (14); pAA1191, which contained a 1.1-kb *Bgl* II *URA3* fragment replacing a 320-bp *Bgl* II fragment of *YTA10* (M. Rep and L. A. Grivell, personal communication); pPT45, which contained a 1.5-kb *Sma* I *URA3* fragment replacing a 1.37-kb *Hpa* I fragment of *YME1* (17); and pG25/ST7, which contained a 1.1-*Kpn* I *URA3* fragment replacing a 400-bp fragment of *RCA1* (A. Tzagoloff, personal communication).

PCR and Sequencing. The sequences of the *cyc1* mutations were verified by DNA sequencing of the pertinent PCR-generated segments. The *cyc1* transformants were subcloned, and genomic DNA was prepared from a single colony by lysing the cells in 100 μl of water at 100°C for 15 min and precipitating cellular debris with 50 μl of 5 M potassium acetate at 4°C for 1 hr. Supernatant, containing the DNA for PCR, was collected after centrifugation at 15,000 × *g* for 5 min. The region containing the *cyc1* mutation of each transformant was amplified by the PCR and sequenced by the dideoxy chain-termination method (20) using the Sequenase dGTP DNA sequencing kit (United States Biochemical).

Low-Temperature Spectroscopic Examination and Spectrophotometric Recordings of Intact Cells. Total amounts of cytochrome *c* were determined by spectroscopic visual examination of intact cells at −196°C (21) and by comparing the intensities of the c_α bands at 547 nm to the c_α bands of strains having known amounts of cytochrome *c*. The levels of cytochromes $a{\cdot}a_3$, *b*, *c*, and c_1 were quantitatively estimated by absorbency recordings of intact cells at −196°C with an Aviv model 14 spectrophotometer as described by Hickey *et al.* (22). The relative amounts of cytochrome *c* were estimated from the heights of the c_α band after baseline corrections.

Pulse–Chase Labeling of Cells and Immunoprecipitation of Cytochrome *c*. Each strain was grown to stationary phase in YPD (1% yeast extract/2% Bacto-peptone/2% dextrose), and the cells were resuspended in 12 ml of a semisynthetic sulfate-free medium (23) lacking yeast extract and containing 2% raffinose and 0.1% glucose. After the cells were incubated for 40 min at 30°C, [^{35}S]methionine (1300 Ci/mmol, Amersham) was added to a concentration of 0.125 Ci/ml, and the cells were incubated for an additional 10 min, followed by a chase in 30 mM (final concentration) methionine. The effectiveness of the addition of unlabeled methionine in terminating labeling was confirmed by demonstrating that the radioactivity of the trichloroacetic acid precipitates did not increase in the cultures during the chase period. Identical volumes of cells were taken at the times indicated. The cells were lysed by the procedure of Yaffe and Shatz (24). A total of 2 ml of cell suspensions was lysed in an equal volume of 0.4 M NaOH and 1.7% 2-mercaptoethanol. After 10 min on ice, 100 μl of 100% trichloroacetic acid was added to precipitate the cells. After 10 min on ice, each sample was centrifuged at 15,000 × *g* for 10 min, the supernatant was removed, and 5 ml of acetone was added. Samples were recentrifuged at 15,000 × *g* for 10 min. The pellets were rinsed once more in acetone, allowed to dry, and subsequently solubilized by boiling for 5 min in a solution of 2% (wt/vol) SDS/0.15 M Tris·HCl, pH 7.5/1 mM EDTA/5% 2-mercaptoethanol/2 mM phenylmethylsulfonyl fluoride. These solubilized samples were centrifuged at 15,000 × *g* for 5 min, and the pellet was discarded. Immunoprecipitation was carried out as described by Dumont *et al.* (25). Each sample was mixed with 10 ml of TNET [1% (wt/vol) Triton X-100/0.14 M NaCl/1 mM EDTA/50 mM Tris·HCl, pH 8.0]. The sample was then mixed with 300 μl of serum and incubated with shaking at 4°C overnight. A total of 1 ml of a 10% (vol/vol) suspension of protein A-coated *S. aureus* cells was added, and the samples were incubated with shaking at room temperature for 1 hr, followed by centrifugation at 1000 × *g* for 15 min. The cells and bound antigen were washed three times in TNET, once in 0.1% SDS/10 mM Tris·HCl, pH 8.0/2 mM EDTA/0.025% sodium azide, and then once more in TNET. A total of 200 μl of 2× loading buffer (4% SDS/0.125 M Tris·HCl, pH 6.8/20% glycerol/10% 2-mercaptoethanol/0.002% bromophenol blue) for SDS/polyacrylamide gel electrophoresis was added to each sample, and the mixture was incubated in boiling water for 5 min. Portions were loaded onto a 10% SDS/polyacrylamide gel optimized for separation of small fragments (26). The antibodies used for immunoprecipitation were prepared essentially as described by Matner and Sherman (27).

RESULTS AND DISCUSSION

Lower Amounts of Iso-2 and Iso-1 in ρ^- Mutants. In an early study by Downie *et al.* (7), 18 of 62 *cyc7-H1* mutants were found to have a greater deficiency in ρ^- strains compared with ρ^+ strains. In extreme cases, iso-2 in the *cyc7-H1-54, cyc7-H1-56*, and *cyc7-H3-26* strains, for example, was at the 100% *CYC7-H1* or *CYC7-H3* levels, respectively, in ρ^+ strains but was completely or almost completely deficient in ρ^- strains.

PCR amplification and sequencing of the *CYC7* translated region of these mutants revealed that the *cyc7-H1-54* and *cyc7-H1-56* mutants contained a G41R replacement, whereas the *cyc7-H3-26* mutant contained a T78I replacement (vertebrate cytochrome *c* numbering system).

To generalize and extend these findings, we have introduced equivalent replacements in iso-1 by transforming strains directly with synthetic oligonucleotides. The results with the T78I iso-1 and G41R iso-1 mutants, presented in Fig. 1 and Table 1, were approximately equivalent to the results with the iso-2-cytochromes *c*. In this genetic background, there was a slight diminution of iso-1 in the *CYC1*$^+$ ρ^- strain, compared with the corresponding *CYC1*$^+$ ρ^+ strain. The *cyc1-1195* ρ^+ and *cyc1-1233* ρ^+ strains contained, respectively, 60% and 45% of the normal iso-1 level, but none could be detected in ρ^- strains derived from these ρ^+ strains.

Lower Amounts of Iso-1 in Mutants Deficient in Cytochromes $a{\cdot}a_3$ and c_1. The lack of specific cytochromes causing the deficiency of the abnormal iso-1-cytochromes *c* was further defined with a series of congenic mutants having disrupted nuclear genes or having a single-site mitochondrial mutation. The results, summarized in Table 1, clearly indicate that the T78I and G41R iso-1 deficiencies in ρ^- strains can be simply explained by the lack of cytochrome $a{\cdot}a_3$, but not by the lack of cytochrome *b*. Furthermore, the *cyt1*-Δ strain demonstrated that the lack of cytochrome c_1 also caused a deficiency of T78I iso-1. Thus, the two physiological partners, cytochromes $a{\cdot}a_3$ and c_1 are required for preservation of the T78I iso-1 mutant.

Altered Forms of Iso-1 Are Labile. The deficiency in abnormal iso-1 can be attributed to diminished biosynthesis or enhanced degradation of any of a number of components in the cytochrome *c* biosynthetic pathway. We have tested the possibility that the iso-1 deficiencies were due to enhanced degradation by employing the Asn-52 → Ile (N52I) replacement, which acts as a "global" suppressor *in vivo* by functionally correcting the defects caused by at least Gly-6 → Ser (G6S), Gly-29 → Ser (G29S), and His-33 → Pro (H33P) replacements (29, 30). In addition, the N52I replacement thermodynamically stabilizes *in vitro* the otherwise normal iso-1 as well as mutant forms (31). The results with the N52I

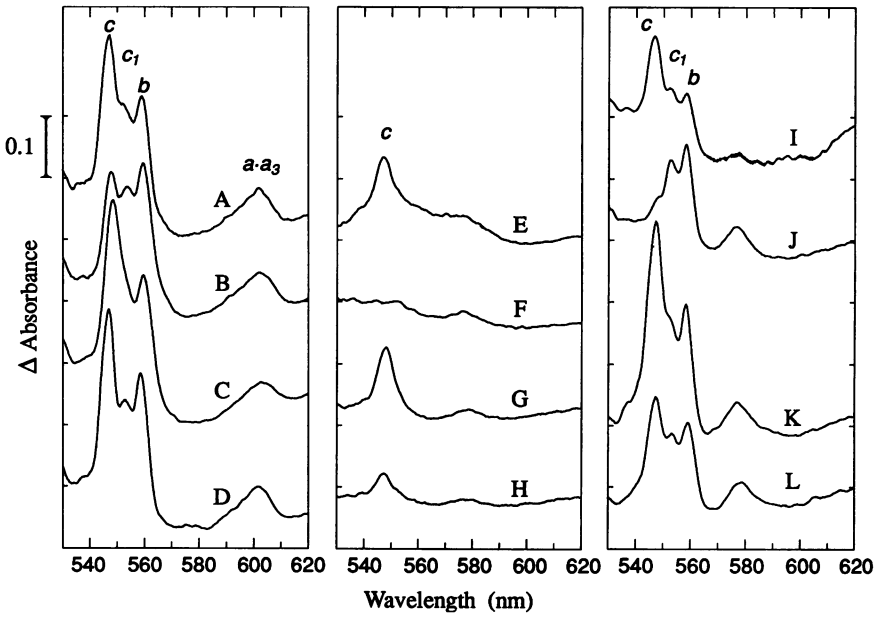

FIG. 1. Low temperature (−196°C) spectrophotometric recordings of a series of congenic yeast strains. The strains, described in Table 1, were grown on 1% sucrose (28) at 30°C for 3 days, and the absorption spectra were recorded as described (22). The α peaks of cytochromes $a{\cdot}a_3$, *b*, c_1, and *c* are located, respectively, at 602.5, 558.5, 553.3, and 547.3 nm. Curves: A, B-8514 (*CYC1*$^+$ ρ^+); B, B-8513 (*cyc1-1195* ρ^+), T78I; C, B-8611 (*cyc1-1196* ρ^+), N52I; D, B-8610 (*cyc1-1197* ρ^+), T78I N52I; E, B-8516 (*CYC1*$^+$ ρ^-); F, B-8515 (*cyc1-1195* ρ^-), T78I; G, B-8613 (*cyc1-1196* ρ^-), N52I; H, B-8612 (*cyc1-1197* ρ^-), T78I N52I; I, B-8607 (*CYC1*$^+$ *cox7*-Δ ρ^+); J, B-8606 (*cyc1-1195 cox7*-Δ ρ^+), T78I; K, B-8615 (*cyc1-1196 cox7*-Δ ρ^+), N52I; L, B-8614 (*cyc1-1197 cox7*-Δ ρ^+), T78I N52I. All of these congenic strains also contained the Cys-102 → Ala replacement, which prevents protein–protein dimerization after extraction required for *in vitro* studies. The peak at 577 nm, seen at various levels in curves E–L, is due to zinc protoporphyrin. The apparent diminished α peak of cytochrome c_1 in *cyc1-1196* ρ^+ (curve C) is due to masking by the higher level of cytochrome *c*. The slight shoulder at ≈547 nm in the *cyc1-1195 cox7*-Δ ρ^+ strain (curve J) may not be due to a low amount of cytochrome *c*, because a similar shoulder is observed in *cyc1*-Δ *cyc7*-Δ ρ^+ strains, which are completely deficient in cytochrome *c*. The *cox7*-Δ and *pet122*-Δ strains had identical spectra.

T78I series of mutants (*cyc1-1197*) shown in Table 1 demonstrated that the N52I replacement alleviated the deficiencies of the T78I iso-1 caused by the lack of cytochromes $a{\cdot}a_3$ and c_1. In addition, pulse–chase experiments directly demonstrated that the deficiency of the T78I iso-1 was due to degradation (Fig. 2). No appreciable turnover was detected after 2 hr with the normal iso-1, the T78I N52I iso-1, and the N52I iso-1. Based on a previous study, we estimate that the half-life of these stable forms was >7 hr. In contrast, the T78I iso-1 appeared completely degraded after 1 hr, having a half-life of <30 min. Thus, we suggest that these labile forms of iso-1 and iso-2 having T78I or G41R replacements are degraded by a mitochondrial protease(s) unless they are protected by cytochromes $a{\cdot}a_3$ and c_1.

There are still other replacements, in addition to T78I and G41R, that cause preferential degradation in ρ^- strains. Also, the study of Downie *et al.* (7) revealed that some mutants were deficient in both the ρ^+ and ρ^- state, and only a subclass showed greater deficiency in the ρ^- strains. Furthermore, not all labile cytochromes *c* are more rapidly degraded in ρ^- mutants. In fact, a systematic study of ρ^+ and ρ^- strains having various replacements of G6 did not reveal any pronounced preferential diminutions in ρ^- strains, and most ρ^+ and ρ^- pairs were deficient to approximately the same extent. For example, the G6A replacement (*cyc1-1113*) caused iso-1 to be thermodynamically unstable (32), and both strains B-8298 (*cyc1-1113* ρ^+) and B-8824 (*cyc1-1113* ρ^-) contained ≈50% of the normal level of iso-1. Furthermore, pulse–chase experiments revealed that iso-1 in both the *cyc1-1113* ρ^+ and *cyc1-1113* ρ^- strains were labile (Fig. 3), having half-lives of, respectively, ≈2 hr and <2 hr. Although the pulse–chase experiments suggested that G6A iso-1 in the ρ^- strain was slightly more labile than the iso-1 in the ρ^+ strain, these results are in marked contrast with the finding that T78I iso-1 in the ρ^+ strain did not appear to be degraded, similar to normal iso-1, but was rapidly degraded in the ρ^- strain. The G6A N52I iso-1 in both ρ^+ and ρ^- strains were more stable, having half-lives of >2 hr. Thus, only a subclass of labile iso-1-cytochromes *c* are significantly protected by cytochrome $a{\cdot}a_3$ and presumably by cytochrome c_1.

Table 1. Levels of iso-1 with various amino acid replacements in strains having various deficiencies of cytochromes $a{\cdot}a_3$, *b*, or c_1

Pertinent genotype	Cytochrome			Iso-1 levels in strains with wild-type and mutant *CYC1* alleles, %				
	a·3	*b*	*c*1	*CYC1*$^+$ (None)	*cyc1-1195* (T78I)	*cyc1-1197* (T78I N52I)	*cyc1-1196* (N52I)	*cyc1-1233* (G41R)
[ρ^+]	+	+	+	100	60	100	100	45
[ρ^-]	0	0	+	75	0	50	75	0
[ρ^+] *cox7*-Δ	0	+	+	100	0	100	100	0
[ρ^+] *pet122*-Δ	0	+	+	100	0	100	100	0
[ρ^+] *cyt1*-Δ	+	+	0	100	0	100	100	—
[*cob*$^-$]	+	0	+	75	45	45	85	—

The genotype of the mitochondrial DNA is denoted in square brackets. The amino acid replacements encoded by the *CYC1* alleles are in parentheses.

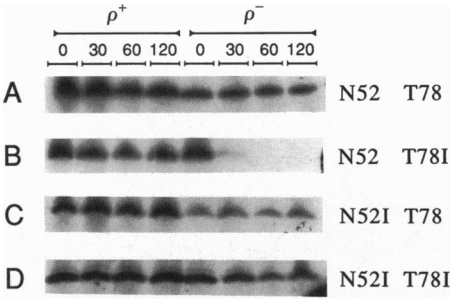

FIG. 2. Pulse–chase labeling of iso-1 from ρ^+ and ρ^- congenic yeast strains containing T78 and N52 and their replacements. After the chase in unlabeled methionine, identical volumes of cell suspensions were taken at the various times, indicated in min at the top of the figure. (*A*) Normal, T78 N52, B-8514 (*CYC1*$^+$ ρ^+) and B-8516 (*CYC1*$^+$ ρ^-). (*B*) T78I, B-8513 (*cyc1-1195* ρ^+) and B-8515 (*cyc1-1195*ρ^-). (*C*) N52I, B-8611 (*cyc1-1196* ρ^+) and B-8613 (*cyc1-1196* ρ^-). (*D*) T78I N52I, B-8610 (*cyc1-1197* ρ^+) and B-8612 (*cyc1-1197* ρ^-). Lower levels of labeling were consistently observed with ρ^- and *pet* strains, because of the diminished growth under derepressed conditions.

The Protease Responsible for Degrading Labile Forms of Isocytochromes *c* Is Unknown. A number of genes encoding, or presumably encoding, mitochondrial proteases were disrupted, and the levels of iso-1 were determined in ρ^+ and ρ^- strains having either the *cyc1-1195* or *CYC1*$^+$ alleles. If these proteases are responsible for the degradation of the T78I iso-1 mutant and other labile forms of iso-1, the disruptions should act as suppressors in ρ^- strains.

The nuclear gene *PIM1* encodes an ATP-dependent protease located in the mitochondrial matrix (14, 33). Because Pim1 is in the mitochondrial matrix and cytochrome *c* in the intermembrane space, Pim1 is not expected to be the protease acting on the labile forms of iso-1. On the other hand, *YTA10* (18), also denoted *AFG3* (17), encodes a protease that acts on incompletely synthesized polypeptides in the mitochondrial inner membrane (34). *YME1* (16) and *RCA1* (19), also denoted *YTA11* and *YTA12*, respectively (18), encode putative proteases related to Yta10 and represent members of a family of ATPases similar to proposed proteolytic complexes in *Escherichia coli* and yeast.

None of the disruptions, *pim1*-Δ, *yta10*-Δ, *yme1*-Δ, and *rca1*-Δ, increased the level of iso-1 in the *cyc1-1195* ρ^- strains. Therefore, the protease responsible for degrading labile forms of the isocytochromes *c* remains to be determined, but it could correspond to other putative proteases recently uncovered (18).

Protection by Protein–Protein Interactions? The highly preferential degradation of certain labile forms (T78I and G41R) but not others (G6A) suggests that either the site on the cytochrome *c* molecule or the nature of the abnormal conformation plays a role in protection by cytochromes $a{\cdot}a_3$ and c_1. Because both cytochromes $a{\cdot}a_3$ and c_1 are required for protection of labile forms, one can suggest that cytochrome *c* is in close proximity to both of these cytochromes during the redox process.

The results obtained with these certain labile iso-1 and iso-2-cytochromes *c* are reminiscent of the results obtained with certain *hem1* mutations, which apparently encode labile forms of mitochondrial δ-aminolevulinic acid synthetase that are deficient in ρ^- and [*oxi2*] strains but not in ρ^+ strains (35, 36). The δ-aminolevulinic acid synthetase deficiencies were more pronounced when the yeast was grown in glucose medium (36).

Also, the level of normal cytochrome c_1 is slightly diminished in ρ^- mutants having certain genetic backgrounds (37). It is reasonable to suggest that this diminution is due to enhanced degradation in the ρ^- condition because of the lack of interaction with a mitochondrial component.

Furthermore, the lack of both iso-1 and iso-2 leads to the loss of cytochrome $a{\cdot}a_3$; *cyc1*-Δ *cyc7*-Δ strains lacking both iso-1 and iso-2 and *cyc3*-Δ strains lacking cytochrome *c* heme lyase (and therefore all cytochromes *c*) concomitantly lack cytochrome $a{\cdot}a_3$ (2, 38). As observed with the certain *hem1* mutants (36), certain "leaky" *cyc3* mutants caused greater cytochrome $a{\cdot}a_3$ deficiencies when the strains were grown on glucose medium (39). It is reasonable to suggest that the mitochondrial protease responsible for these degradations may be enhanced under glucose-repressed conditions. Nevertheless, these results suggest to us that cytochrome *c* may be protecting the normal cytochrome $a{\cdot}a_3$ from degradation. Thus, the cytochromes could be mutually protecting themselves from degradation.

The phenomenon described in this study superficially resembles the findings that strongly interacting components of several protein complexes are resistant to degradation, whereas an excess of one component is readily degraded, as exemplified with ribosomal proteins from yeast (40–42). However, it remains to be seen if these two phenomena are truly related.

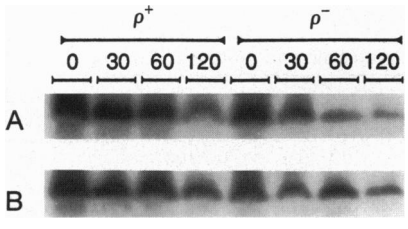

FIG. 3. Pulse–chase labeling of iso-1 from ρ^+ and ρ^- congenic yeast strains containing G6 and N52 and their replacements. (See the Fig. 2 legend.) (*A*) G6A N52, B-8298 (*cyc1-1113* ρ^+) and B-8824 (*cyc1-1113* ρ^-). (*B*) G6A N52I, B-8293 (*cyc1-1127* ρ^+) and B-8843 (*cyc1-1127* ρ^-).

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